

## Responses of dark respiration in the light to desiccation and temperature in the intertidal macroalga, *Ulva lactuca* (Chlorophyta) during emersion

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Dark respiration (nonphotorespiratory mitochondrial CO<sub>2</sub> release) in the light ( $R_L$ ) of the intertidal macroalga *Ulva lactuca* (Chlorophyta) during emersion was investigated with respect to its response to variations in temperature and desiccation.  $R_L$  was estimated by CO<sub>2</sub> gas-exchange analysis using the Kok effect method, whereas dark respiration in darkness ( $R_D$ ) was determined from CO<sub>2</sub> release at zero light. Rates of  $R_L$  were significantly and consistently lower than those of  $R_D$  in emersed *U. lactuca* across all the temperature and desiccation levels measured. This demonstrated that dark respiration was partially depressed in the light, with the percentage inhibition ranging from 32 to 62%. Desiccation exerted a negative effect on  $R_L$  and  $R_D$  at a high temperature, 33°C, whereas it had much less effect on respiration at low and moderate temperatures, 23 and 28°C. In general,  $R_L$  and  $R_D$  increased with increasing temperature in *U. lactuca* during all stages of emersion but responded less positively to temperature change with increasing desiccation. Additionally, the  $Q_{10}$  value (i.e. the proportional increase of respiration for each 10°C rise in temperature) for  $R_L$  calculated over the temperature range of 23 to 33°C was significantly higher than that for  $R_D$  in *U. lactuca* during the initial stages of emersion. Respiratory carbon loss as a percentage of gross photosynthetic carbon gain increased with increasing temperature and/or desiccation but was significantly reduced when estimated using  $R_L$  rather than  $R_D$ . It is suggested that measurements of  $R_L$  and how it changes in a variable environment are as important as estimates of  $R_D$  and photosynthesis in determining simultaneous balance between photosynthetic carbon uptake and respiratory carbon loss and in modeling the net daily carbon gain for an intertidal macroalga.

KEY WORDS: Dark respiration, Desiccation, Emersion, Intertidal macroalgae, Kok effect, Temperature, *Ulva*

### INTRODUCTION

Dark respiration (nonphotorespiratory mitochondrial CO<sub>2</sub> release) is a pivotal metabolic pathway producing usable energy (ATP) and reductants [NAD(P)H], as well as carbon skeleton intermediates to support other metabolic processes during plant growth and maintenance (Krömer 1995; Amthor 2000; Atkin *et al.* 2005). It has been well documented that plants respire roughly half of their daily photosynthetic carbon gain (Ryan 1991; Amthor 2000). Therefore, dark respiration plays an essential role in determining the carbon budget of individual plants. Dark respiration occurs both in the light and in the darkness. Specifically, dark respiration that occurs in the light can modulate stromal redox balance during the photosynthetic process (Foyer & Noctor 2000). It may also aid in minimizing the production of potentially damaging reactive oxygen species through reoxidizing the excess cellular redox equivalents generated by chloroplasts under excess irradiance conditions (Saradadevi & Raghavendra 1992; Purvis 1997; Maxwell *et al.* 1999). Moreover, mitochondrial respiratory activity during illumination provides ATP for the repair of photosynthetic proteins (in particular the D1 protein of PSII) degraded by photoinhibition, protecting against photoinhibitory damage of the photosynthetic apparatus (Raghavendra *et al.* 1994; Atkin *et al.* 2000b). Mitochondrial respiration therefore functions in light during photosynthesis, and it affects and is affected by

the potential demands for this process to supply energy and carbon skeletons during hours of illumination (Hoefnagel *et al.* 1998; Shapiro *et al.* 2004).

Dark respiration in the light ( $R_L$ ) has usually been assumed to be of the same magnitude as in darkness in most studies of the carbon balance of plants or plant organs (e.g. Graham 1980; Pooter *et al.* 1990; Collier *et al.* 1991; Zou & Gao 2002, 2003, 2005). However, there is growing evidence that  $R_L$  in terrestrial higher plants varies between 25 and 100% of dark respiration in darkness ( $R_D$ ), indicating that light partially inhibits dark respiration in photosynthetic tissue (Sharp *et al.* 1984; Kirschbaum & Farquhar 1987; Brooks & Farquhar 1985; Villar *et al.* 1994, 1995; Krömer 1995; Atkin *et al.* 2000b; Wang *et al.* 2001; Shapiro *et al.* 2004). The inhibition of dark respiration in the light is even evident at irradiances as low as 3–50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Sharp *et al.* 1984; Brooks & Farquhar 1985; Atkin *et al.* 1998), regardless of the light quality (red, blue or white; Atkin *et al.* 1998). This inhibition appears to be a result of photosynthetic products acting as regulators of respiratory function (McCashin *et al.* 1988; Wang *et al.* 2001; Tovar-Mendez *et al.* 2003). It has been suggested that the mitochondrial pyruvate dehydrogenase complex is reversibly phosphorylated during illumination, which eventually influences the CO<sub>2</sub> efflux through the Krebs cycle (Budde & Randall 1990; Gemel & Randall 1992; Tovar-Mendez *et al.* 2003). Additionally, the mitochondrial isocitrate dehydrogenase was shown to be inhibited by the

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high ratios of NADPH/NADP occurring during illumination (Igamberdiev & Gardeström 2003).

Intertidal macroalgae, being the important source of primary production in intertidal zone, are exposed to the atmosphere periodically resulting from tidally driven emersion–immersion cycles. This aerial exposure can last several hours at night as well as during the daytime. Emerged intertidal macroalgae suffer pronounced variations of potentially stressful environmental conditions (such as tissue water loss, increased irradiance and varied thallus temperature) as tissues dry with respect to submersion, which can bring about direct physiological effects on them (e.g. Dring & Brown 1982; Johnston & Raven 1986; Madsen & Maberly 1990; Bell 1993; Matta & Chapman 1995; Davison & Pearson 1996; Beach & Smith 1997; Peña *et al.* 1999).

There exists considerable information on the ability of intertidal macroalgae to photosynthesize during periods of exposure. It appears that not only intertidal algae tolerate the emerged conditions and recover their photosynthetic activity following resubmersion but also the carbon fixation during emersion contributes significantly to the daily carbon balance of the plants (Madsen & Maberly 1990; Bell 1993; Beach & Smith, 1997; Peña *et al.* 1999; Kawamitsu *et al.* 2000). Although diffusion boundary layers are usually thicker in air relative to water, this constraint on inorganic carbon supply is more than offset by a diffusion coefficient for CO<sub>2</sub> in air that is 10,000-fold higher than in water (Madsen & Maberly 1990; Raven 1997, 1999). Therefore, emersion may facilitate inorganic carbon flux at the macroalgal surface and thereby elevate carbon fixation rates, even for species able to utilize HCO<sub>3</sub><sup>-</sup> in photosynthesis (Beer & Eshel 1983; Holbrook *et al.* 1988; Madsen & Maberly 1990; Raven 1999). For example, some intertidal macroalgae have been demonstrated to exhibit higher photosynthetic rates under emerged conditions than under submerged conditions, and photosynthesis can be enhanced when partially desiccated (e.g. Johnson *et al.* 1974; Quadir *et al.* 1979; Johnston & Raven 1986; Gao & Aruga 1987; Einav & Beer 1993; Lipkin *et al.* 1993; Einav *et al.* 1995). However, the advantage in inorganic carbon acquisition by use of CO<sub>2</sub> in air rather than aqueous inorganic carbon is offset as water loss from the thallus. Decreased photosynthesis as a result of desiccation during emersion is a major reason why the time spent emerged is not more productive regarding net carbon assimilation (Raven 1997, 1999). Macroalgae possess no anatomical features analogous to the stomata or impermeable cuticles of terrestrial vascular plants that may effectively protect against water loss (exceptions are the saccate algae; Oates 1985, 1986), and thus cannot avoid desiccation during emersion at low tide (see review by Lüning 1990). However, photosynthesis is much more tolerant to desiccation in intertidal macroalgae than in terrestrial vascular plants because of differences in the molecular environment around the photosynthetic enzymes (Madsen & Maberly 1990; Kawamitsu *et al.* 2000) and the capacity of recovering photosynthesis within only 1–2 h following severe desiccation in intertidal macroalgae (Pearson *et al.* 2000). Tolerance to desiccation in air had been thought to be an important physiological requirement for macroalgae growing high up in the intertidal (e.g. Dring & Brown 1982; Beer & Kautsky 1992; Peña *et al.* 1999).

Although there is a large literature detailing the photosyn-

thetic responses of intertidal macroalgae to emersion-related environmental factors (see review by Davison & Pearson 1996 and references therein; Beach & Smith 1997; Harker *et al.* 1999; Kawamitsu & Boyer 1999; Peña *et al.* 1999; Zou & Gao 2002, 2003), respiratory responses to changing environmental conditions are much less well understood (Madsen & Maberly 1990; Romaine *et al.* 1997; Zou & Gao 2002) in spite of the importance of dark respiration in determining the instantaneous daily carbon balance and algal metabolism. In particular, to our knowledge, there is no report focusing on the extent to which changes of temperature and cellular water content influence mitochondrial respiration that takes place during the hours of illumination in intertidal macroalgae when exposed to air during low tides. Achieving a better knowledge of the mechanistic response of R<sub>L</sub> to the environmental variables associated with emersion has the potential to estimate more precisely the instantaneous or daily carbon balance in intertidal macroalgae. In the present study, we focused on the dark respiration in an intertidal macroalga during emersion. We have attempted to address two specific questions during emersion cycles using the intertidal green macroalga *Ulva lactuca* as the experimental material: (1) Is R<sub>L</sub> lower than R<sub>D</sub> in common with the light inhibition of dark respiration occurring in many species of terrestrial higher plants? (2) How are R<sub>L</sub> and the proportion of R<sub>L</sub> to gross photosynthesis at saturating light (A<sub>gross</sub>) modified by temperature and desiccation in emerged *U. lactuca*? *Ulva* species (Chlorophyta) are commonly found along the intertidal and subtidal regions throughout the world. *Ulva lactuca* is one of the most common *Ulva* species found in the shoreline of Nanao Island, Shantou, China. It thrives in the mid- to low intertidal zone and therefore spends a significant fraction of its time out of the seawater during the tidal cycles.

## MATERIAL AND METHODS

### Plant materials and laboratory maintenance

Thalli of *U. lactuca* L. were collected at low tide from the middle intertidal zone of Nanao Island, Shantou, China, in May 2005. Collected algae were gently rinsed and cleaned of sediments and epiphytes. They were placed into a plastic barrel with some natural seawater and were transported to the laboratory within 3 h. The algae were then maintained in filtered natural seawater (salinity c. 32) in plexiglass aquaria under about 180 μmol photons m<sup>-2</sup> s<sup>-1</sup> (PAR, LD cycle 12 h : 12 h) and 28 ± 0.5°C (corresponding to the ambient surface seawater temperature at the site of collection). The seawater was continuously aerated and renewed every day. The algal samples were used for experiments within 3 days of collection. After this period, the algae remains were discarded and fresh samples recollected. The ambient surface seawater temperature at the site of collection over the sampling period in May 2005 was between 27.5 and 28.5°C. Such a small change in seawater temperature did not have a significant influence on the temperature–response curves of the algal samples.

### CO<sub>2</sub> gas exchange measurement

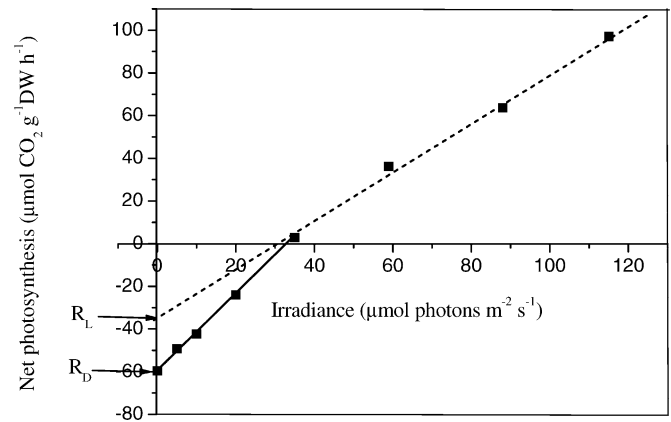
Although it is easy to measure R<sub>D</sub>, measurements of R<sub>L</sub> are not straightforward as a result of the presence of other instan-

taneous processes of CO<sub>2</sub> exchanges in the light such as glycine decarboxylation and RuBP carboxylation (Villar *et al.* 1994; Atkin *et al.* 1998). There are two commonly used approaches to estimate  $R_L$  based on gas-exchange techniques: the Kok method (Kok 1948) and the Laisk method (Laisk 1977). The Laisk method, described by Laisk (1977) and extended by Brooks & Farquhar (1985), measures photosynthesis at low internal CO<sub>2</sub> concentrations and varying irradiances. Given that at such low CO<sub>2</sub> concentrations where CO<sub>2</sub> fixation and photorespiration are balanced, the rate of CO<sub>2</sub> release stand for  $R_L$ . As pointed out by Villar *et al.* (1994), the main shortcoming of this approach is that the analysis must be carried out at a very low CO<sub>2</sub> concentration that is far from the normal growth conditions. In addition, it is not known whether there is a direct short-term effect of CO<sub>2</sub> concentrations on  $R_L$  (Shapiro *et al.* 2004).

We therefore chose the Kok method, rather than the Laisk method, to estimate  $R_L$  in the present experiments. The Kok method analyzes the response of net rate of photosynthesis over low irradiances and can be measured at normal CO<sub>2</sub> conditions. At very low levels of irradiance, the response is linear, and the slope (i.e. the photosynthetic efficiency) is relatively steep, but near the light compensation point (the irradiance level at which net photosynthesis is zero), there is an abrupt break in the linear response, and the slope decreases. This change, termed the 'Kok effect', has been attributed to an increase in the respiration rate resulting from a gradual disappearance of the light-induced inhibition of dark respiration (Kok 1948; Sharp *et al.* 1984; Villar *et al.* 1994). The linear section of the light curve above the break extrapolates to an estimate of  $R_L$  (Villar *et al.* 1994; Krömer 1995; Wang *et al.* 2001), while the line at irradiance below the break stretches to  $R_D$ , which is determined at zero irradiance.

Photosynthetic rates of the emersed *U. lactuca* thalli were measured as CO<sub>2</sub> exchange in an open-flow gas-exchange system, using an infrared gas analyzer (LCA-4, Analytical Development Co) at ambient atmospheric CO<sub>2</sub> concentrations (c. 360 ppm). The light source was a metal halide lamp (220/240 V, 150 W, Hikaric-J) suspended above the photosynthetic leaf chamber. Temperature was controlled by maintaining the chamber in a temperature-controlled cabinet. About 1.5 g fresh weight (initial wet weight) of algal material were spread out in the photosynthetic chamber, and the dry weight (DW) was determined after each experiment by oven drying (80°C for 24 h). Net photosynthesis ( $P_n$ ) or dark respiration ( $R$ ) [ $\mu\text{mol CO}_2 \text{ g(DW)}^{-1} \text{ h}^{-1}$ ] was calculated as follows:  $P_n$  (or  $R$ ) =  $\Delta C \cdot F \cdot 60 \cdot 273 / [(273 + T) \cdot 22.4 \cdot \text{DW}]$ , where  $\Delta C$  is the difference in CO<sub>2</sub> concentration (ppm) between the inlet and outlet air;  $F$  is the gas flow rate (L/min),  $T$  is temperature (°C), and DW is the dry weight (g).

Eight irradiances between 0 and 120  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  were used to analyze the photosynthetic responses at low irradiance. Irradiance was adjusted by altering the distance between the light source and the photosynthetic chamber.  $R_L$  was estimated by the method described above. A representative light-response curve for emersed *U. lactuca* at low irradiance is presented in Fig. 1.  $R_D$  was determined at zero irradiance by turning off the light source and covering the chamber with a black cloth. In addition, the light-saturated maximum net photosynthesis ( $A_{\text{max}}$ ) for each algal sample case was mea-



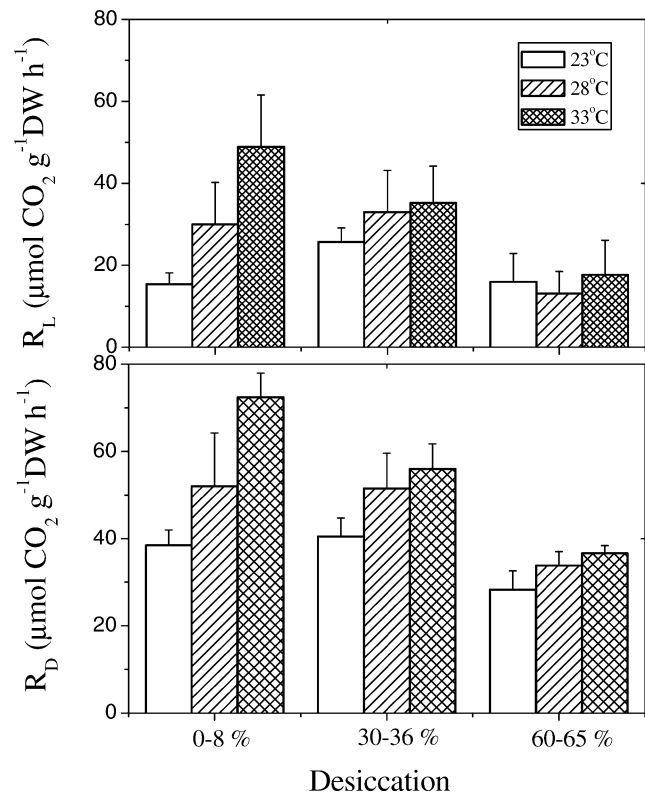
**Fig. 1.** A representative photosynthetic light-response curve of *Ulva lactuca* during emersion at low irradiance. Temperature was 28°C, and the percent desiccation of thalli was 0–8%. The linear regression section of light-response curve before the distinct break in the slope extrapolated back to the Y axis, and the intercept was given an estimate of dark respiration in the light ( $R_L$ ), according to the Kok method. The value for the dark respiration in darkness ( $R_D$ ) was taken at zero irradiance.

sured at irradiance of 600  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , which saturated photosynthesis without causing photoinhibition.

Photosynthetic response to irradiance was measured under three temperatures (23, 28 and 33°C) and three levels of desiccation (thallus water loss of 0–8%, 30–36% and 60–65%) in an effort to analyze the effects of those variables on  $R_L$  and  $R_D$ . Initially, thalli submersed in seawater were acclimated to a single measuring temperature for 30 min. Algae were then emersed and desiccated in an incubator at 160  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , 75  $\pm$  5 % relative humidity and the same temperatures as used for initial acclimation and photosynthesis measurement. Samples were weighed at regular intervals through the various desiccation regimes in order to obtain the desired levels of desiccation. The percent desiccation (D %) was estimated as follows:  $D \% = (W_o - W_t) / (W_o - W_d) \cdot 100$ , where  $W_o$  is the initial wet weight (i.e. fully hydrated weight) measured after removing superficial water from the thalli by gently blotting with tissue paper,  $W_t$  is the desiccated weight after a known time interval and  $W_d$  is the dry weight (80°C for 24 h). We sorted the samples in three classes of desiccation (desiccation 0–8%, 30–36% and 60–65%) in terms of the levels of water loss measured in the present study. These classes of desiccation were arbitrarily designated as low, medium and high, respectively. When the above measurements had been carried out at one temperature, the procedure was then repeated at a new measurement temperature, using similar algal thalli that had not previously been subjected to the measurement protocol. The order of temperature treatments (28, 23 and 33°C) was randomly assigned.

$Q_{10}$  values (i.e. the proportional change in dark respiration per 10°C rise) were calculated for  $R_L$  and  $R_D$  over the measured temperature interval of 23 to 33°C using the following equation according to Atkin *et al.* (2000a):  $Q_{10} = 10^{(\text{slope} \cdot 10)}$ , where the slope is the regression slope of a log<sub>10</sub>-transformed respiration rate (either  $R_L$  or  $R_D$ ) vs temperature plot. As pointed out by Atkin *et al.* (2000a), this would reduce the chances of erroneous  $Q_{10}$  values coming from unusually high or low respiration values at each of the low and high measuring tem-





**Fig. 2.**  $R_L$  and  $R_D$  of emersed *Ulva lactuca* subjected to different conditions of temperature and thalli water loss. Vertical bars represent  $\pm$  standard deviation of the means ( $n = 3\text{--}6$ ).

peratures used in the study. We calculated a single  $Q_{10}$  value using mean rates of respiration of all replicates.

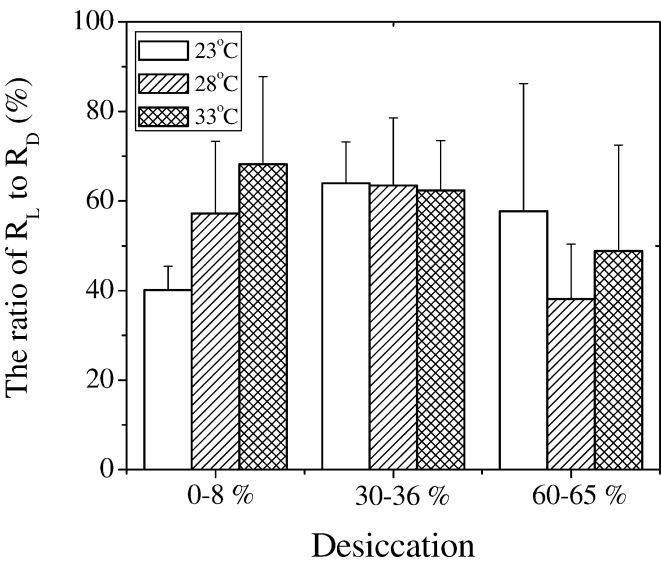
The data plotted on graphs were mean values with standard deviations ( $s$ ) and were analyzed with one- and two-way analysis of variance and Student's  $t$  test by using SPSS for Windows software package version 10. Differences were considered to be significant if  $P < 0.05$ .

RESULTS

The values of  $R_L$  and  $R_D$  of *U. lactuca* during emersion under different temperature and thalli desiccation conditions are shown in Fig. 2. It was evident that  $R_L$  values were significantly and consistently lower ( $P < 0.01$ ) than  $R_D$  across all the temperature and desiccation levels measured, demonstrating that dark respiration was partially inhibited in the light. The ratio of  $R_L$  to  $R_D$ , which reflected the magnitude of inhibition of dark respiration by light, ranged from a low of 38% to a maximum of 68% (Fig. 3). However, no consistent trend in the  $R_L/R_D$  ratio was clear under the various combinations of temperature and desiccation conditions.

There was a decreasing trend in the rates of both  $R_L$  and  $R_D$  with increasing desiccation at 33°C (Fig. 2). However, for algae incubated at 23 and 28°C, mild desiccation (30–36%) did not effect ( $P > 0.1$ ) either  $R_L$  or  $R_D$ . A positive effect of the mild desiccation was even observed for  $R_L$  at 23°C.

In general, both  $R_L$  and  $R_D$  increased with increasing measurement temperature from 23 to 33°C during all the stages of emersion, with the exception that  $R_L$  remained unchanged



**Fig. 3.** The ratio of  $R_L$  to  $R_D$  of emersed *Ulva lactuca* subjected to different conditions of temperature and thalli water loss. Vertical bars represent  $\pm$  standard deviation of the means ( $n = 3\text{--}6$ ).

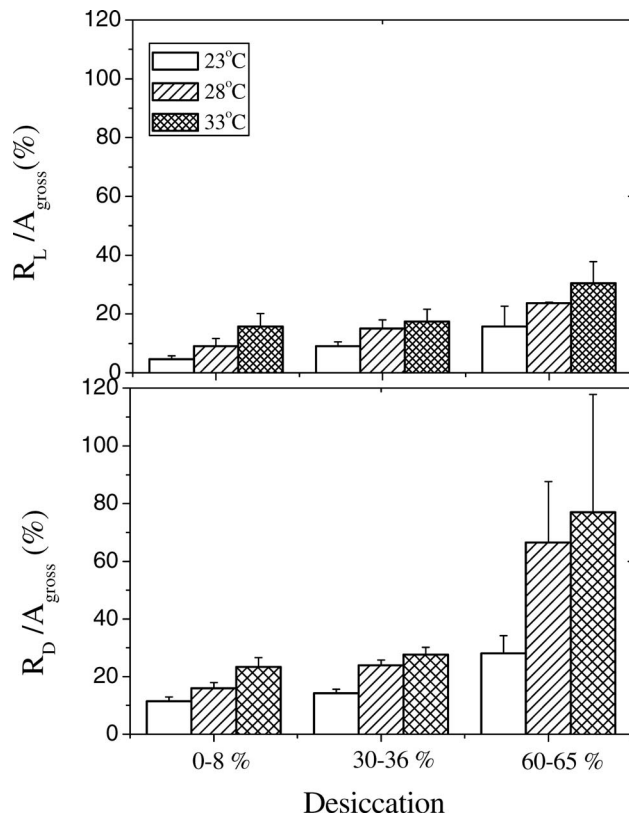
with the rise of temperature in highly desiccated algae (Fig. 2). Temperature had a smaller effect on  $R_L$  and  $R_D$  with increasing water loss from algal thalli (Fig. 2), which was further evident from the change of the  $Q_{10}$  values (Table 1). The  $Q_{10}$  values, calculated over the measured temperature interval of 23 to 33°C, were reduced from 3.17 to 1.11 or from 1.88 to 1.29, respectively, for  $R_L$  and  $R_D$  with the increasing degree of desiccation. It was evident that  $R_L$  and  $R_D$  differed significantly in their relative response to temperature during the initial stages of emersion. The higher  $Q_{10}$  value of  $R_L$  (3.17) relative to that of  $R_D$  (1.88) in low desiccated algae may have been a consequence of the small absolute value of  $R_L$  determined at low temperature (23°C), which thereby amplified the relative difference in  $R_L$  over the measured temperature range.

A large variability was found in respiratory carbon loss ( $R_L$  or  $R_D$ ) as a percentage of gross photosynthetic carbon gain ( $A_{\text{gross}}$ , i.e.  $A_{\text{max}} + R_L$ ) in *U. lactuca* when emersed and subjected to various combinations of temperature and desiccation (Fig. 4).  $R_L$  and  $R_D$  were 4.6–30.4% and 11.4–77.1% of light-saturating gross photosynthetic carbon gain, respectively. A consistent trend was clear that both  $R_L : A_{\text{gross}}$  and  $R_D : A_{\text{gross}}$  ratios increased with increasing temperature and/or increasing degree of desiccation. Additionally, the ratios of  $R_L : A_{\text{gross}}$  were substantially and consistently lower than those of  $R_D : A_{\text{gross}}$  across all the combinations of temperature and desiccation measured.

**Table 1.**  $Q_{10}$  values for  $R_L$  and  $R_D$  of emersed *Ulva lacutuca* subjected to varying degree of desiccation.<sup>1</sup>

|                    | Desiccation |        |        |
|--------------------|-------------|--------|--------|
|                    | 0–8%        | 30–36% | 60–65% |
| $Q_{10}$ for $R_L$ | 3.17        | 1.37   | 1.11   |
| $Q_{10}$ for $R_D$ | 1.88        | 1.38   | 1.29   |

<sup>1</sup> Values are based on Fig. 2.



**Fig. 4.** Percentage of  $R_L$  and  $R_D$  to gross photosynthesis at saturating light ( $A_{\text{gross}}$ , i.e.  $A_{\text{max}} + R_L$ ) of emersed *Ulva lactuca* subjected to different conditions of temperature and thalli water loss. Vertical bars represent  $\pm$  standard deviation of the means ( $n = 3-6$ ).

## DISCUSSION

To our knowledge, it was often assumed in previous studies of carbon balance in macroalgae that dark respiration during illumination continued at the same magnitude as that in darkness. However, the present results showed that  $R_L$  was consistently and significantly lower than  $R_D$  in the common intertidal macroalga *U. lactuca* during emersion over all measured temperatures and levels of desiccation. This demonstrated that dark respiration was repressed in the light, with the percentage inhibition of respiration in the light ranging from 32 to 62%. This finding was consistent with reports for many species of terrestrial higher plants (e.g. Villar *et al.* 1994, 1995; Atkin *et al.* 1997, 1998, 2000, 2006; Wang *et al.* 2001; Shapiro *et al.* 2004). The present results underline the importance of using  $R_L$  rather than  $R_D$  to more accurately estimate the proportion of photosynthetically fixed carbon that is respired and to model the total amount of carbon gained during the hours of illumination in such intertidal macroalga as *U. lactuca*.

The mechanism of light inhibition of dark respiration remains debatable and appears to be complex. The physiological explanation for the inhibition of dark respiration by light had been ascribed to the inhibiting effect of the light on the respiratory enzymes regulated by the accumulation of photosynthetic metabolites during the hours of illumination, such as NADPH and ATP (McCashin *et al.* 1988; Krömer 1995; Tovar-Mendez *et al.* 2003), which is suggested to down-reg-

ulate the respiratory pathway in the light regarding both glycolysis and the Krebs cycle. Recently, Tcherkez *et al.* (2005) reported the metabolic basis of inhibition by light of leaf respiration *in vivo* study by feeding experiments using  $^{13}\text{C}$ -enriched substrates and followed the  $^{13}\text{C}$  atoms with isotope ratio mass spectrometry and nuclear magnetic resonance. Their results indicated that metabolic down-regulation (glycolysis, Krebs cycle) accompanies the light/dark transition and emphasized the decrease of Krebs cycle decarboxylations as a metabolic basis of the light-dependent inhibition of mitochondrial respiration. Our results of the inhibition of respiration in the light might suggest that emersed *U. lactuca* had a lower demand for respiratory products such as energy and carbon skeletons during the illumination hours than during darkness periods.

Temperature has fundamental influences on chemical reaction rates. It is viewed as one of the most important environmental parameters affecting the rates of respiration in plants, with the temperature sensitivity of respiration reflecting the effects of temperature on enzyme activity, adenylate control (i.e. ADP/ATP ratios) and/or substrate supply (Atkin & Tjoelker 2003; Atkin *et al.* 2005). During emersion at low tide, intertidal macroalgae usually experience changes in temperature. The present experiments showed that, although both  $R_L$  and  $R_D$  responded positively to the increasing measurement temperature in emersed *U. lactuca*, increasing water loss from thalli resulted in a reduced temperature sensitivity for both  $R_L$  and  $R_D$ , which was reflected in the decreased value of  $Q_{10}$  (the proportional increase in respiratory rate for each  $10^\circ\text{C}$  rise) for both  $R_L$  and  $R_D$  with increasing desiccation. For algae subjected to low desiccation, the calculated  $Q_{10}$  value for  $R_D$  was 1.9, being similar to the value (2.0) often assumed for dark respiration in plants (Ryan 1991; Tjoelker *et al.* 2001). However, the  $Q_{10}$  value for  $R_L$  (3.2) was considerably higher than that for  $R_D$  in algae with low desiccation. This indicated that  $R_L$  responded much more positively to increasing temperature than  $R_D$  did in algae during the initial stages of emersion.  $Q_{10}$  values more than 3.0 have also been reported in many species of terrestrial higher plants (e.g. Atkin & Tjoelker 2003; Shapiro *et al.* 2004).

*Ulva lactuca* is highly susceptible to desiccation during emersion owing to its thin sheet-like thallus morphology. Desiccation might affect reactions catalyzed by enzymes located at the water/membrane interface such as ATPase or soluble enzymes (Kaiser 1987). Excessive water loss could also lead to increased surface pH, resulting in reduction or cessation of photosynthesis (Bidwell & McLachlan 1985). While the photosynthetic responses to emersion-related desiccation in intertidal macroalgae have been an area of intensive investigation (see review by Davison & Pearson 1996 and references therein; Beach & Smith 1997; Harker *et al.* 1999; Kawamitsu & Boyer 1999; Peña *et al.* 1999; Zou & Gao 2002, 2003), the responses of dark respiration to desiccation have received much less attention. Information available shows that, although increasing desiccation would decrease the rate of dark respiration, the rate of decline is usually lower than that of photosynthesis (Madsen & Maberly 1990; Zou & Gao 2002). The results in the present study also agree with this finding (the photosynthetic data not shown) since intense desiccation exerted a less deleterious influence on respiration (either  $R_L$  or  $R_D$ ) than on photosynthesis. It was also suggested that the

decline of respiration in emersed *U. lactuca* was not simply due to the reduced photosynthesis (which thereby might reduce the supply of respiratory substrate) but to other effects of desiccation *per se* because the reduced photosynthesis was not necessarily accompanied with the reduced respiration. However, the biochemical mechanism underpinning the reduced respiration in intense desiccated algae is waiting to be investigated. Additionally, it is important to note that the relation of dark respiration and desiccation in emersed *U. lactuca* was temperature dependent. The rate of decline of either  $R_L$  or  $R_D$  resulting from more intense desiccation was reduced remarkably with decreasing temperature. Moreover,  $R_L$  and  $R_D$  exhibited different response patterns to desiccation. At low temperature (23°C), high desiccation exerted no negative effect on  $R_L$ , whereas it reduced the rate of  $R_D$  by 26.3%. In contrast, the percentage decrease of respiration caused by greater desiccation was greater in  $R_L$  (64 %) than in  $R_D$  (50%) when the thalli were incubated at high temperature (33°C).

A large variability in percentage dark respiration relative to light-saturating rates of gross photosynthesis observed in this experiment in emersed *U. lactuca* might reflect the varying physical conditions of desiccation and temperature, indicating that variations in the physical conditions affected the carbon balance. Two important trends could be seen when evaluating the instantaneous photosynthetic carbon uptakes and respiratory carbon losses during daytime emersion. On the one hand, substituting  $R_L$  for  $R_D$  substantially increased estimates of the percentages of carbon gained to carbon lost in *U. lactuca* across all the combinations of temperature and desiccation. It is therefore proposed that estimates of net carbon balance during illumination for an emersed macroalga based on  $R_L/A_{\text{gross}}$  ratios might be more appropriate than estimates based on  $R_D/A_{\text{gross}}$  ratios. On the other hand, the percentages of carbon respired via  $R_L$  relative to gross carbon gained at  $A_{\text{max}}$  increased appreciably with the increasing temperature and/or desiccation, implying a decreasing balance between net carbon gain and desiccation or temperature.

The response pattern to temperature in dark respiration usually differs from that in photosynthesis (Davison 1991; Atkin & Tjoelker 2003). For example, unlike photosynthesis, which displays the optimum temperature of up to approximately 5–10°C higher than the ambient seawater temperature, respiratory rates in marine macroalgae can continue to increase with increasing temperature of seawater in excess of 40°C (Zou & Gao 2005). In the present experiments, a decreasing trend of light-saturated net photosynthesis in emersed *U. lactuca* was observed with increasing temperature over the tested range of 23–33°C (data not shown), suggesting the optimum temperature of photosynthesis by emersed *U. lactuca* to be 23°C or lower. By contrast, both  $R_L$  and  $R_D$  showed an increasing trend with increasing temperature. On the other hand, either  $R_L$  or  $R_D$  was much less sensitive to water loss than was photosynthesis, which can presumably be attributed to respiratory processes (such as the activities of respiratory enzymes in glycolysis and the Krebs cycle and respiratory electron transport chain) being less water status sensitive than the photosynthetic processes (such as photosynthetic electron transfer and the enzymatic reactions of the Calvin cycle). Collectively, our data that dark respiration (either  $R_L$  or  $R_D$ ) exhibited a different temperature and/or desiccation sensitivity compared with light-saturated photosynthesis may supply the physiological

interpretation for the observed increasing percentage of respiratory carbon loss relative to photosynthetic carbon fixation. Our results have important implications for accounting for the simultaneous budget between photosynthetic carbon fixation and respiratory carbon loss.

In conclusion, the results obtained in the present experiments indicated that  $R_L$  values in emersed *U. lactuca*, when measuring  $\text{CO}_2$  gas exchange using the Kok method, were significantly and consistently lower than  $R_D$  values. This demonstrated that dark respiration is partially inhibited in the light in emersed *U. lactuca*, which is consistent with the findings obtained from many species of terrestrial higher plants. The difference in  $R_L$  and  $R_D$  emphasized the necessity of substituting  $R_L$  for  $R_D$  when trying to accurately estimate mitochondrial respiration sustained during illumination. We therefore proposed that measurements of  $R_L$  and how it changes in a variable environment are as important as estimates of  $R_D$  and photosynthesis in determining the simultaneous balance between photosynthetic carbon uptake and respiratory carbon loss and in modeling the net daily carbon gain for a macroalga.

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